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PATENT TRADEMARK OFFICE

Docket No: 2094/1E286US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Jeffrey M. LINNEN and Kevin M. GORMAN

Serial No.: 09/493,353 Art Unit: 1655

Filed: January 28, 2000 Examiner: J. GOLDBERG

For: OLIGONUCLEOTIDE PRIMERS FOR EFFICIENT DETECTION OF HEPATITIS C VIRUS (HCV) RNA AND METHODS OF USE THEREOF

DECLARATION OF KEVIN M. GORMAN UNDER 37 C.F.R. § 1.132

Hon. Commissioner of Patents and Trademarks
Washington, DC 20231

Sir:

I, Kevin M. GORMAN, hereby declare and state as follows:

1. I am a citizen of the United States of America and am more than 21 years of age.

2. I presently hold the position of Research Scientist at Wyeth-Lederle Vaccines where I have been employed since September, 1999. Prior to this position, I held the position Research Scientist at Ortho-Clinical Diagnostics, Inc. (an affiliate company of Johnson & Johnson and the Assignee of the above-captioned patent application). My qualifications are set forth more fully on the copy of my *Curriculum Vitae* attached hereto at Exhibit Tab 1.

3. I am one of the named inventors in the above-identified patent application.

4. I have read and am familiar with the instant application as it was filed in the U.S. Patent and Trademark Office (the "USPTO"). I have been advised by counsel, and therefore believe, that this application is based on and benefits from the earlier filing date of the prior provisional patent application Serial No. 60/118,497 filed on February 3, 1999.

5. I have also read and am familiar with the pending claims of the application as amended August 17, 2001. I have been advised by counsel, and therefore believe, that among the pending claims in this application are ones directed to polymerase chain reaction ("PCR") assays, including reverse transcription PCR ("RT-PCR") assays that use certain oligonucleotides to reverse

transcribe and/or amplify hepatitis C virus ("HCV") nucleic acids in a sample. I further understand, based on the advice of counsel, that other pending claims in this application are directed to particular oligonucleotides which may be used in such assays and to kits which may be used to practice such an assay.

6. I have also been advised by counsel, and therefore believe, that the pending claims have been rejected by the USPTO as being obvious over combinations of the following references:

(a) Han *et al.*, "Characterization of the terminal regions of hepatitis C viral RNA: Identification of conserved sequences in the 5' untranslated region and poly(A) tails at the 3' end" *Proc. Natl. Acad. Sci. U.S.A.* 1991, 88:1711-1716 ("Han");

(b) Kolykhalov *et al.*, "Identification of a Highly Conserved Sequence Element at the 3' Terminus of Hepatitis C Virus Genome RNA" *J. Virology* 1996, 70(6):3363-3371 ("Han");

(c) U.S. Patent No. 5,837,463 issued November 17, 1998 to Tanaka *et al.*, ("Tanaka");

(d) Encke *et al.*, "Total Chemical Synthesis of the 3' Untranslated Region of the Hepatitis C Virus with Long Oligodeoxynucleotides" *J. Virological Methods* 1998, 74:117-121 ("Encke");

(e) U.S. Patent No. 5,846,704 issued December 8, 1998 to Maertens *et al.* ("Maertens"); and

(f) Ahern, "Biochemical Reagent Kits Offer Scientists Good Return on Investment" *The Scientist* 1995, 9(15):20 ("Ahern").

I have read and am familiar with each of these references.

7. Described herein are certain experiments relating to the oligonucleotides and methods that are recited in the pending claims of this application. The experiments were carried out by my co-inventor and myself, or by others working under our supervision and control. The results from these experiments demonstrate that, at the very best, persons similarly skilled in the art of PCR amplification could have only been motivated to *try* using particular oligonucleotides to amplify HCV nucleic acids. In particular and as demonstrated by the experiments described here, when this application was first filed (*i.e.*, as of the February 3, 1999 priority date) it was not possible to predict *a priori* whether a particular primer pair would successfully amplify HCV nucleic acids, *e.g.*, in a clinical sample.

8. More specifically, the experiments described here relate to assays that use RT-PCR to detect HCV nucleic acids in a sample. In these assays, an oligonucleotide sequence that is complementary to some genomic sequence of

HCV is first used to prime the reverse transcription of HCV RNA molecules in the sample. The complementary DNA obtained by that reaction is then amplified, using other oligonucleotides as primers for a polymerase chain reaction (PCR).

9. In the experiments described here, forward and reverse primers were designed to specifically hybridize to and thereby amplify a particular region of the HCV genome: the 3' non-coding (NC) region. At the time these experiments were performed, the 3' NC region had been described and was already known. See, *e.g.*, the Kolykhalov reference discussed in ¶ 6(b), above. My co-inventor and I decided to try the experiments described here to see if particular oligonucleotides we had derived from this region might successfully prime the reverse transcription of representative HCV genotypes and thereby provide greater sensitivity for detecting those genotypes, *e.g.*, in clinical samples. However, before performing these experiments we did not know which of the primer pairs, *if any*, would work in such an assay.

10. In a first series of experiments, four reverse PCR primers were designed and tested for their ability to amplify HCV nucleic acids in a sample. These primers included a primer described in the present application and referred to therein as 57R27 (SEQ ID NO:9).[1] The other primers tested are referred to as

[1] The sequence identification numbers (SEQ ID NOS) used in this Declaration correspond to those used to identify the corresponding oligonucleotide sequence in

66R25, 67R25 and 72R27. Each of the primers tested in this set of experiments is listed in Table I, below, along with its nucleotide sequence. For those primers that are also described in this application, the corresponding Sequence Identity Number is also provided in Table I. All of these primers were designed to by complementary to, and thereby reverse transcribe, the 3' NC region of HCV.

TABLE I

HCV-specific primers

designation	sequence	seq id no.	comment(s)
57R27	5'-AGGCCAGTATCAGCACTCTCTGCAGTC-3'	9	reverse PCR primer
66R25	5'-TGCAGAGAGGCCAGTATCAGCACTC-3'	N/A	reverse PCR primer
67R25	5'CTGCAGAGAGGCCAGTATCAGCACT-3'	N/A	reverse PCR primer
72R27	5'-ACATGATCTGCAGAGAGGCCAGTATCA-3'	N/A	reverse PCR primer
1F27	5'-GGTGGCTCCATCTTAGCCCTAGTCACG-3'	8	forward PCR primer

11. To evaluate their performance, each reverse primer shown in Table I was first used to reverse transcribe HCV nucleic acids in a clinical sample. The reverse transcription products were then amplified using the same reverse PCR primer, and detected on an ethidium bromide gel. In more detail, a clinical HCV sample was obtained from Boston Biomedica, Inc. (West Bridgewater, Massachusetts) with the viral load having already been estimated by the supplier.

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the instant patent application.

The sample was divided into aliquots containing an estimated 50 copies of viral RNA for each reaction (1000 copies/ml) and each aliquot was reverse transcribed using the above-described reverse primers and following the protocols set forth in this application (see, in particular, the Reverse Transcription Methods described on page 13 of the application as filed). The resulting cDNA products were then PCR amplified according to the protocol set forth on page 14 of the application as filed. The forward primer 1F27[2] was added to each sample immediately prior to running the PCR reaction, whereas the primer used to reverse transcribe the viral RNA was left to serve as the reverse PCR primer. The resulting amplification products were detected on ethidium bromide gels according to standard protocols.

12. When combined with the forward primer 1F27, the reverse primer 72R27 gave very little or no gel bands even after 40 cycles of PCR indicating that this primer completely failed to reverse transcribe the HCV nucleic acids. The band obtained using the reverse primer 66R25 was somewhat more intense than that obtained with the reverse primer 67R25. However, both of these bands appeared as broad smears on the ethidium bromide gels, and not as sharp, distinct bands which are preferable, *e.g.*, for clinical applications. By contrast, the

[2] This PCR primer is also described in the patent application as filed, and corresponds to SEQ ID NO:8 in that application's Sequence Listing. For convenience, the 1F27 primer's nucleotide sequence is also set forth in Table I, *supra*.

combination of forward primer 1F27 (SEQ ID NO:8) and reverse primer 57R27 (SEQ ID NO:9) yielded robust, clean bands.

13. These results demonstrate that, of the PCR primers tested, only the combination of forward primer 1F27 (SEQ ID NO:8) and reverse primer 57R27 (SEQ ID NO:9) successfully amplifies HCV nucleic acid with sufficient sensitivity and specificity for use, *e.g.*, in a clinical assay.

14. I declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true. I further declare that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the instant application or of any patent issued thereupon.

Respectfully submitted,

Dated: 4/17/02

Kevin M. Gorman

Exhibit (Tab 1: *Curriculum Vitae* for Kevin M. GORMAN)

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